Molecular Biology

FUNCTIONAL ANALYSIS OF THE G450 AND C483 MUTATIONS IN *ESCHERICHIA .COLI* 16 S RIBOSOMAL RNA

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Protein production in all organisms is catalyzed by ribosomes. Ribosomes are composed of ribosomal RNA (rRNA), which is now believed to be the catalytic moiety, and ribosomal proteins (rproteins), which are believed to facilitate proper folding of the rRNA. In *E. coli*, the 70 S ribosome is composed of 50 S and 30 S subunits. The 30S subunit contains 16S rRNA and 21 rproteins. The 50 S subunit contains 23 S rRNA, 5 S rRNA and 34 rproteins.

This study focuses on mutations in the 16 S rRNA at nucleotides G450 and C483 in the conserved Helix 17 (H17) structure. H17 is present in the ribosomes of specific groups of bacteria but absent in eukaryotes. In ribosome crystal structures, G450 and C483 form a Watson-Crick pair that separates two internal loops in H17. The goal of the project is to determine if base pairing between these nucleotides is functionally important and if so, what role H17 plays in protein synthesis.

To achieve the goal, single and double site-directed mutations were constructed at these sites using a genetic system developed in the Cunningham lab at Wayne State University. In this system, plasmid-encoded ribosomes specifically translate two reporter genes; the green fluorescent protein (GFP) gene and chloramphenicol acetyltransferase (CAT) gene. Specific expression of the CAT gene allows genetic selection experiments and GFP provides for high-throughput analysis of ribosome function in living cells. Our results show that base-pairing and not nucleotide identity between positions 450 and 483 of 16S rRNA is essential for ribosome function. These data suggest that pairing between G450 and C483 facilitates folding of H17 into a functionally active conformation.